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Transport of pyruvate into the mitochondrion of *Trypanosoma brucei*

Jitka Štáfková[a], Jan Mach[a], Fréderic Bringaud[b], Jan Tachezy[a]
[a]*Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic*
[b]*Centre de Résonance Magnétique des Systèmes Biologiques, UMR5536, Université de Bordeaux, CNRS, Czech Republic*
E-mail: jitka.stafkova@natur.cuni.cz

Trypanosoma brucei is a pathogen of livestock and humans transmitted by tse-tse flies in sub-Saharan Africa. Different life-cycle stages of trypanosomes present adaptations to their specific environment. In bloodstream and procyclic *T. brucei*, these include changes to mitochondrial morphology and function, and overall metabolic rearrangements reflected by different spectra of metabolic end products. In bloodstream *T. brucei*, ATP is generated primarily by glycolysis, pyruvate being the predominant excreted product of metabolism. In contrast, procyclic-stage *T. brucei*, found in the midgut of the insect vector where glucose is scarce, depend on mitochondrial catabolic pathways for ATP production. Proline and threonine are candidate carbon sources for these stages. In vitro, these are eventually metabolized to succinate, acetate and glycine. Regulating the availability of pyruvate in the mitochondrion is one of the modes of balancing oxidative phosphorylation and glycolysis; in bloodstream *T. brucei* this balance is shifted heavily towards glycolysis. We seek to determine whether *T. brucei* transports pyruvate into the mitochondrion using a mitochondrial pyruvate carrier homologous to the one recently identified in fruit fly, human and yeast cells (MPC). In addition, we address the relative importance of the pyruvate transporter in procyclic and bloodstream trypanosomes. To this end, we identified two MPC homologs in the genome *T. brucei* and confirmed the mitochondrial localization of the epitope-tagged proteins in both procyclic and bloodstream stages. We generated MPC1 knock-out cell lines in both these stages, showing that the pyruvate transporter is dispensable for *T. brucei* under standard culture conditions. The adaptations of mitochondrial metabolism in this model, as documented by end product analysis using HPLC and NMR, let us comment on mitochondrial metabolism in *T. brucei* in general.

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Role of formate channel coding focA and focB genes in H_2 production by *Escherichia coli* upon glucose fermentation at slightly alkaline pH

Karen Trchounian[a], Varduhi Abrahacpbmyan[a], Anait Vassilian[a], Armen Trchounian[b]
[a]*Yerevan State University*
[b]*Department of Microbiology, Plants & Microbs Biotechnology, Faculty of Biology, Yerevan State University, Armenia*
E-mail: k.trchounian@ysu.am

Escherichia coli is able to encode four [Ni–Fe] hydrogenases (Hyd) having different roles in H_2 production and oxidation during glucose and/or glycerol fermentation that is dependent on pH [1]. Moreover, *E. coli* has two formate channels coded by focA and focB genes which are situated at the end of hyc and hyf operons, respectively. FocA has an important role in regulating intracellular formate level during anaerobic fermentation [2]. Role of focB gene is not clear. As end product of mixed-acid fermentation, formate is exported from the cytoplasm to periplasm where, in the presence of terminal electron acceptors, it serves as a substrate of the periplasmic formate dehydrogenases. In the absence of terminal electron acceptors it is re-imported to the cytoplasm to produce H_2. H_2 evolving activity in focB mutant grown on glucose, in glucose supplemented assays, at pH 7.5 was determined to be ~1.6 fold lower than in wild type. No difference was detected for focA mutant. To understand the role of formate in wild type H_2 producing activity, in the assays external formate at concentration of 10 mM was added. In the assays with formate H_2 evolving activity of focA mutant was similar, but in focB mutant it was ~2 fold lower than in wild type. Taken together these results have shown FocB activity during glucose fermentation in both assays. Moreover, no role of FocA in H_2 producing activity was observed. This might be due to that formate would be imported into the cell via FocB but not FocA channel. The low level of H_2 production rate in focB mutant in glucose and formate assays would result in sufficiently lower level of formate production in cytoplasm and due to some interaction between foc and pyruvate formate lyase (pfl) genes in the transcriptional level; the latter has been also suggested by the other group [2].

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The critical role of Na^+/Ca^{2+} exchanger on the maintenance of T-tubule structure

Yoshihiro Ujihara[a], Satoshi Mohri[a], Yuki Katanosaka[b]
[a]*Department of Physiology, Kawasaki Medical School, Japan*
[b]*Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okaya, Japan*
E-mail: ujihara@med.kawasaki-m.ac.jp

Cardiac Na^+/Ca^{2+} exchanger (NCX1) is the primary Ca^{2+} extrusion system in beating myocytes, essential for Ca^{2+} homeostasis, and important in Ca^{2+} handling during excitation–contraction (E–C) coupling. NCX1 is specially localized in T-tubule and can be close to dyad, where is a predominantly E–C coupling take place. Therefore, T-tubule disorganization is linked to decreased contractility in heart failure (HF). Despite of T-tubule remodeling be correlated with Ca^{2+} handling defects in failing hearts (FH), the molecular mechanism has remained unclear. To examine whether the alteration of NCX1 expression and activity relate to the disorganization of T-tubule structure in FHs, we generated novel transgenic mice expressing NCX1 cardiac-specifically and inducibly, and examined the effect of inducing NCX1 expression during the progression of HF. We followed changes in NCX1 activity and expression during HF progression over 16 weeks in these mice, after transverse aortic constriction (TAC)-surgery. In TAC hearts, NCX1 activity increased over the first few weeks, but started to drop from 8 weeks after TAC before the onset of T-tubule disorganization and myocyte contractile dysfunction, which are common features in failing myocytes. Over the progression of HF, the expression of junctophilin-2 located at T-tubule/sarcoplasmic reticulum (SR) junction was gradually reduced in TAC hearts. Inducing NCX1 expression